ON THE QUESTION OF THE IDENTITY OF SOYBEAN "LIPOXIDASE" AND CAROTENE OXIDASE[+]

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It has been previously reported by Kies that controlled heating of a partially purified soybean lipoxidase causes the differential loss of its capacity to destroy carotene in the coupled reaction with methyl linoleate, but does not affect its capacity to promote the peroxidation of linoleic acid. The catalysis of the coupled reaction has long been ascribed to lipoxidase. It is now shown that the homogeneous crystalline preparation of soybean lipoxidase of Theorell and co-workers, which is effective against linoleic acid, is essentially ineffective in the above mentioned coupled reaction and is further resistant to the heat treatment employed. The catalysis of the coupled reaction must therefore be ascribed to a heat sensitive entity which is distinct from the Theorell lipoxidase.

A carotene-destroying enzyme was found in soybeans in 1928 by Bohn and Haas (1). Four years later Andre and Hou (2) discovered that soybeans contained an enzyme, which they termed "lipoxidase", that oxidized unsaturated fats. Subsequently Sumner and Sumner (3) reported that "lipoxidase" was

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identical with the "carotene oxidase" of Bohn and Haas and demonstrated that lipoxidase acted by peroxidizing unsaturated fat. They also showed that the bleaching of the carotene occurred concurrently with the peroxidation. Theorell, Holman and Åkeson (4) succeeded in crystallizing lipoxidase from soybeans and proved their enzyme to be a single homogeneous protein (E.C. 1.13.1.13; trivial name "lipoxygenase"). A variety of assay methods were employed in the several laboratories where lipoxidase investigations were being carried out, *inter alia*, rate of carotene destruction, rate of peroxide formation, and rate of appearance of conjugated diene. While not all of the methods were equally satisfactory, it was generally accepted that they reflected the activity of a single entity.

The identity of the two activities, "lipoxidase" and "carotene oxidase", was, however, brought into question by one of us (M.W.K. (5)) when it was discovered that "carotene oxidase" activity (assayed by the method of Balls *et al*. (6)) in a partially purified soybean lipoxidase preparation was destroyed by heating at 70° for two minutes, without a corresponding loss of peroxidizing action on linoleic acid (measured essentially by the method of Theorell *et al*. (4)). The differential heat stabilities of the two activities was repeatedly verified by one of us (7).

Although Theorell and his co-workers routinely measured the activity of their crystalline lipoxygenase by following peroxide formation and appearance of conjugated diene absorption at 234 nm, they apparently did not report testing their enzyme for "carotene oxidase" activity in the coupled carotene-methyl linoleate reaction. We have recently succeeded in obtaining a homogeneous crystalline lipoxygenase from soybeans (8) which appears to be identical with that described by Theorell *et al*. (4) on the basis of similar crystalline appearance, molecular weight and general enzymatic properties (8). We have now tested our homogeneous enzyme lipoxidase for both its "carotene oxidase" activity and its "lipoxidase" activity and determined the relative heat stabilities of these activities. As may be seen in the Table, both

Table I: Comparison of Lipoxidase and Carotene Oxidase Activities of the Crystalline Soybean Lipoxidase of Theorell, Holman and Åkeson with Crude Extracts

	Lipoxidase Units	Carotene Oxidase Units	Carotene Oxidase Units / Lipoxidase Units
	per ml	per ml	
Pure Lipoxidase			
Unheated	2.3×10^6	77	3.4×10^{-5}
Heated*	1.8×10^6	77	4.3×10^{-5}
Crude Soybean Extract			
Unheated	1.9×10^4	280	1.5×10^{-2}
Heated*	1.9×10^4	1	-

*Heated for 6 min. at 68°.

Lipoxidase assay: One unit of enzyme causes an increase of absorbancy at 234 nm of 0.001 per min. Substrate: 1 µmole[s] linoleate; 330 µmoles borate buffer, pH 9.0; final volume, 3.0 ml. Temp. 16°.

Carotene oxidase: The method of Balls *et al.* (6) was used with the following modifications. Bleaching was followed in a 10 mm cuvette at 452 nm, in a recording spectrophotometer. One unit of enzyme bleached 50% of the carotene present in the reaction mixture in one minute.

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activities are associated with the pure lipoxidase present and are relatively stable to heating at 68° for 6 minutes. For comparison, results obtained with an unfractionated aqueous extract are shown. In this preparation the "lipoxidase" activity, by the procedure of Theorell *et al.* (4), is stable to heat but the "carotene oxidase" activity, by the procedure of Balls *et al.* (6), is not. It is of special interest that the pure lipoxidase is an exceedingly poor "carotene oxidase" compared to the crude preparation, when results are based upon equal lipoxidase units. Indeed, the crude extract is better by more than two orders of magnitude than the purified enzyme with respect to "carotene oxidase" activity.

It must, therefore, be concluded that the carotene oxidase activity observed in partially purified preparations of soybean extract using the method of Balls et al. (6) cannot be ascribed to the lipoxidase of Theorell et al. (4) but must be due to a second and heat-sensitive enzyme.

In this connection it should be noted that Koch et al. (9) have reported, on the basis of peroxide-forming capacity, the existence of two enzymes in soybeans, differing in their substrate specificity for free and esterified fatty acids. More recently, Guss et al. (10) have demonstrated what appears to be multiple forms of lipoxidase by disc gel electrophoresis and direct enzyme staining.

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